

Bound Volatile Precursors in Genotypes in the Pedigree of 'Marion' Blackberry (*Rubus* Sp.)

XIAOFEN DU,[†] CHAD E. FINN,[‡] AND MICHAEL C. QIAN^{*,†}

[†]Department of Food Science & Technology, Oregon State University, Corvallis, Oregon 97331, and

[‡]Horticultural Crops Research Laboratory, Agricultural Research Service, U.S. Department of Agriculture, Corvallis, Oregon 97330

Glycosidically bound volatiles and precursors in genotypes representing the pedigree for 'Marion' blackberry were investigated over two growing seasons. The volatile precursors were isolated using a C18 solid-phase extraction column. After enzymatic hydrolysis, the released volatiles were analyzed using stir bar sorptive extraction gas chromatography–mass spectrometry (GC-MS) and direct microvial insert thermal desorption GC-MS. The most abundant volatile precursors in the genotypes were alcohols, followed by shikimic acid derivatives. High amounts of furanone glycosides were also detected, while norisoprenoids only existed in a small amount in blackberries. The volatile precursor composition in the genotypes in the 'Marion' pedigree was very similar to their free volatile distribution. 'Logan' and 'Olallie' predominantly had bound norisoprenoids. Wild 'Himalaya' predominated with terpene alcohol and furaneol glycosides, whereas 'Santiam' and 'Chehalem' contained a high level of terpene alcohol glycosides. A similar inheritance pattern was also observed for some volatile precursors in the genotypes in the 'Marion' pedigree. A high content of linalool, hydroxylinalool, and α -ionol glycosides in 'Olallie' and a low content in 'Chehalem' resulted in a moderate level in their offspring 'Marion', while a low content of (*E*)-linalool oxide precursor in 'Olallie' and a high content in 'Chehalem' also resulted in a moderate level in 'Marion'. However, the concentration of furaneol glycosides in 'Marion' exceeded that of its two parents.

KEYWORDS: Blackberry; 'Marion' pedigree; stir bar sorptive extraction (SBSE); solid-phase extraction (SPE); microvial insert thermal desorption

INTRODUCTION

The aroma of fresh fruits comes from volatile compounds generally at concentrations of parts per billion to parts per trillion levels of the fruit weight. However, these trace amounts of aroma-active compounds have a major effect on fruit quality. Many of these volatile compounds are present as free forms and directly contribute to the aroma of the fresh fruits. However, these compounds can also combine with sugars and be stored in the form of odorless glycosides in fruits and plant tissues serving as flavor precursors. The most common glycosides are monosaccharide and disaccharide conjugates, and trisaccharide glycoconjugates also exist (1). Depending on the compounds and the fruits, the occurrence of glycosidic conjugates can be many times greater than the free counterparts (2), making up a huge reserve as aroma potential to fruits. Glycoconjugates of volatile compounds have been found in berries (1–4) and many other fruits (5–10).

Blackberry (*Rubus* subgenus *Rubus* Watson) genotypes have very diverse volatile profiles. Although a large number of volatile and aroma compounds have been identified (11–14), the composition of glycosidically bound volatiles in blackberries is very limited (15). These glycosidically bound volatiles can be released

during fruit processing and consumption and contribute to the overall quality of the fruits.

Fruit aroma is influenced by genotype, environment, and interactions (16). Genotype determines the precursors, enzymes systems, and their activities in flavor formation. As crosses between parents are made in a breeding program, the genes inherited in the offspring from one or both parents, in concert with the environment, will determine the fruit aroma. A limited number of studies have reported the volatile heritability in berry fruits such as raspberry, blueberry, and strawberry (17–22), but very few studies reported the volatile heritability in blackberry (23).

'Marion' is the current industry standard for blackberry quality, especially for flavor quality. The 'Marion' pedigree is complicated with multiple blackberry species and red raspberry in the ancestry and with some genotypes whose parents are unknown. 'Marion' was selected in Oregon from a cross of 'Chehalem' and 'Olallie' blackberries (24). In turn, 'Chehalem' was selected from a cross of 'Santiam' and 'Himalaya' blackberries, while 'Olallie' originates from 'Black Logan', a purported raspberry–blackberry hybrid, and Young.

Volatiles in fruit from genotypes in the 'Marion' pedigree have been studied recently, and some patterns of inheritance have been suggested for the volatile compounds (23). However, the glycosidically bound volatiles and precursors have not been

*To whom correspondence should be addressed. Tel: 541-737-9114. Fax: 541-737-1877. E-mail: michael.qian@oregonstate.edu.

investigated. The objective of this study was to investigate the distribution of glycosidically bound volatiles in the genotypes in the 'Marion' pedigree and to improve our general knowledge about volatile precursor inheritance patterns in fruits.

MATERIALS AND METHODS

Chemicals. Eucalyptol, 2-heptanol, 1-octen-3-ol, 6-methylhept-5-en-2-ol, theaspirane, 1-(2-hydroxy-5-methylphenyl)ethanone, linalool, borneol, citronellol, nopol, hexanoic acid, geraniol, β -ionone, perilla alcohol [(4-prop-1-en-2-yl-1-cyclohexenyl)methanol], octanoic acid, mesifurane, cumic alcohol, eugenol, and isoeugenol were obtained from Aldrich Chemical Co. Inc. (Milwaukee, WI). 1-Hexanol, nerol, benzyl alcohol, and phenylethanol were obtained from Sigma (Milwaukee, WI). Linalool oxide and Furaneol were obtained from Fluka (Buchs, Switzerland). α -Terpineol was obtained from K & K Laboratories (Jamaica, NY). 4-Terpineol, isothymol, and cinnamyl alcohol were obtained from TCI Japan (Tokyo, Japan). *cis*-3-Hexenol was obtained from Bedoukian Research (Danbury, CT). *trans*-2-Hexenol was obtained from Compagnie Parento, Inc. (Lenoir, NC). 1-Heptanol and 1-octanol were purchased from Eastman Chemical (Kingsport, TE). β -Damascenone was obtained from Firmenich (Princeton, NJ). Methyl salicylate and 4-phenylbutan-2-ol were from Alfa Aesar (Ward Hill, MA).

Methanol [high-performance liquid chromatography (HPLC) grade] was from EM Science (Gibbstown, NJ), and dichloromethane (HPLC grade) was from Burdick & Jackson (Muskegon, MI). Standard stock solutions were prepared in methanol individually. Two sets of internal standard were prepared. Internal standard A was composed of eucalyptol, isothymol, γ -heptalactone, and 1-(2-hydroxy-5-methylphenyl)ethanone with concentrations of 3.4, 8.3, 7.6, and 3.5 mg/L; internal standard B was 1-(2-hydroxy-5-methylphenyl) ethanone with a concentration of 70 mg/L.

Anhydrous sodium sulfate (99.9%, ACS certified) was from Mallinckrodt (Mallinckrodt Baker, Phillipsburg, NJ). Fructose, glucose, and citric acid were from Lancaster (Ward Hill, MA); sucrose and malic acid were from Spectrum (Gardena, CA). Synthetic juice containing 3.0% fructose, 3.1% glucose, 0.2% sucrose, 0.8% citric acid, and 0.9% malic acid was prepared according to the procedure described previously (25).

Blackberry Samples. Fully ripe berry samples from 'Marion', 'Chehalem', 'Santiam', 'Himalaya', and 'Olallie' blackberries, 'Logan', a raspberry-blackberry hybrid, and 'Meeker' red raspberry were hand-harvested from plants growing in research plots at Oregon State University Lewis-Brown Farm in Corvallis, Oregon, between June and July of the 2007 and 2008 growing seasons. These cultivars reflected genotypes in the 'Marion' pedigree. 'Meeker' was included as a representative of red raspberry because the original red raspberry cultivars in the 'Marion' pedigree are either not known or not available. While 'Black Logan' is the parent in the 'Marion' pedigree, it has been lost to time and another cultivar from Judge Logan's 19th century breeding program. 'Logan' was included in the study because it is a raspberry-blackberry hybrid, its prominence in the background of many trailing blackberries, and its likely kinship to 'Black Logan'.

The berries were individually quick frozen (IQF) and stored at -18°C until analysis. During analysis, 100 g of IQF berry fruit was thawed in a refrigerator (1°C). With equal weights of distilled water and 1% calcium chloride (final concentration) added, the sample was then blended in a glass jar (Waring Products Div., Dynamics Corp. of America, New Hartford, CO) in high-speed pulse mode for 20 s. The puree was centrifuged for 20 min at 5000 rpm. The supernatant of juice was filtered through a Waterman #1 filter paper (particle retention $> 11\ \mu\text{m}$), followed by a VWR 413 filter paper (particle retention $> 5\ \mu\text{m}$). The filtered clear juice was used for analysis.

Precursor Analysis. Bound glycoside precursors were isolated using BAKERBOND SPE C18 disposable extraction cartridge (500 mg, 6 mL, from J. T. Baker, Deventer, Netherlands) as reported previously (26), with some modification. Each 10 mL of blackberry juice was loaded onto a C18 cartridge preconditioned with 10 mL of methanol followed with 10 mL of distilled water. The cartridge was washed with 20 mL of distilled water, followed by 20 mL of dichloromethane, to remove sugar, organic acids, and free volatiles. The glycoside extracts were eluted from the cartridge into a 40 mL vial with 6 mL of methanol. The eluent was evaporated to dryness in a rotary evaporator at 45°C under vacuum.

Twenty milliliters of citrate buffer solution (0.2 M, pH 3.1) and 100 μL of Macer8 FJ enzyme solution (Biocatalysts Limited, Wales, United Kingdom) were added into the dried glycoside extracts. The mixtures were incubated at 45°C for 12 h. After hydrolysis, the mixtures were cooled to room temperature for either stir bar sorptive extraction (SBSE) or solid-phase extraction (SPE) gas chromatography-mass spectrometry (GC-MS) analysis.

SBSE GC-MS. The released volatile compounds were analyzed using SBSE GC-MS as described previously (23). Each 10 mL of blackberry juice was added to a 20 mL vial, in which 3 g of sodium chloride and 20 μL of internal standard solution (A) were added. A stir bar (Twister) coated with poly(dimethylsiloxane) (PDMS) phase (1 cm length, 0.5 mm thickness, Gerstel Inc., Baltimore, MD) was used to extract volatile compounds. The sample was extracted with the Twister bar for 2 h at a speed of 1000 rpm. GC-MS analyses were performed using an Agilent 6890 gas chromatograph with a 5973 mass selective detector (Agilent, Santa Clara, CA). A programmed temperature vaporizer injector (PTV, Gerstel) in a cooled injection system (CIS4, Gerstel) was used in the GC-MS system. A CIS linear packed with 1 cm of Tenax sorbent (TA, 60/80, Supelco, Bellefonte, PA) was used to trap volatile compounds during thermal desorption of samples.

The TDU has an initial temperature of 25°C . After a sample was loaded, TDU was heated at a rate of $300^{\circ}\text{C}/\text{min}$ to a final temperature of 250°C with 1 min of holding. TDU injection was in splitless mode during thermal desorption, while the PTV was in a solvent vent mode with a venting flow of 60 mL/min for 4.7 min, at a venting pressure of 22.8 psi. After the solvent vent, the PTV was switched to splitless mode for 3.0 min and then changed to split mode with a venting flow of 50 mL/min. The initial temperature of PTV was kept at -80°C for 0.2 min and then ramped at a rate of $10^{\circ}\text{C}/\text{s}$ to a final temperature of 250°C with 10 min of holding.

Compound separation was achieved with a DB-WAX column (30 m \times 0.25 mm i.d., 0.25 μm film thickness, Phenomenex, Torrance, CA). The oven temperature was programmed at 40°C for 2 min of holding and then increased to 230°C at a rate of $4^{\circ}\text{C}/\text{min}$ with 6 min of holding. A constant helium column flow of 2.5 mL/min was used. A column splitter was used at the end of the column, 1 mL/min column flow was introduced to the MS, and the other 1.5 mL/min column flow was vented out. The MS transfer line and ion source temperature were 280 and 230°C , respectively. Electron impact mass spectrometric data from m/z 35–350 were collected using a scan rate of 5.27/s, with an ionization voltage of 70 eV.

A calibration curve was built up for quantitative analysis. The individual stock solution was diluted in synthetic juice to make the first level mixed standard solution, which was then diluted at a 1:9 (v/v) ratio with synthetic juice to obtain a concentration range. Twenty microliters of internal standard was added to the diluted solution and then extracted using a stir bar, as done for the blackberry juice sample. Standard calibration curves were obtained through Chemstation software and were used to calculate the concentrations of volatile compounds in the samples. Triplicate analysis was performed for each sample.

SPE Direct Microvial Insert Thermal Desorption GC-MS. Polar volatile compounds including phenylmethanol, 2-phenylethanol, and Furaneol were determined using a SPE direct microvial insert thermal desorption technique described previously, with some modification (27). Each 10 mL of blackberry juice was passed through a preconditioned Lichrolut-EN cartridge (200 mg, 3 mL, from Merck, Darmstadt, Germany). The cartridge was preconditioned with 5 mL of methanol followed by 10 mL of distilled water. After the sample was loaded, the SPE cartridge was washed with 20 mL of distilled water and then gently dried with air. The retained volatile compounds were eluted with 1 mL of methanol. Twenty microliters of internal standard solution (B) was added, and the eluent was dried with anhydrous sodium sulfate. Ten microliters of the extract was loaded into a 200 μL glass insert and placed into the sample holder of the TDU for GC-MS analysis. The TDU, PTV, and GC-MS conditions were the same as described in the SBSE GC-MS section, except that the TDU was heated at a rate of $100^{\circ}\text{C}/\text{min}$ to the final temperature and the initial PTV temperature was kept at 25°C .

An individual stock solution of phenylmethanol, 2-phenylethanol, and Furaneol was diluted in methanol to make the first level mixed standard solution and then diluted with methanol to a serial concentration. Twenty microliters of internal standard was added to the diluted solution. Each 10 μL of solution was used to build the calibration curves.

Statistical Analysis. The S-PLUS Version 7.0 software (Insightful Corp., Seattle, WA) was used to test the statistical variances of volatile constituents from two growing seasons. A *t* test was conducted to test the growing season variance of each volatile compound. ANOVAs (analyses of variance) were applied for the tests of the variance of each volatile compound among the different cultivars.

RESULTS AND DISCUSSION

Overall, the volatile precursor distribution pattern in the genotypes in the 'Marion' pedigree was similar to the distribution of the free volatile components reported previously (23). The most abundant volatile precursors in the genotypes were lipid derivatives, followed by terpene alcohol glycosides and shikimic acid derivatives. Very high amounts of Furanol glycoside were detected. Norisoprenoid precursors only existed in a small amount in blackberries.

Terpene Alcohol Glycosides and Polyols. Terpene alcohols are mainly present at glycosides, and the most abundant terpene alcohols in the cultivars evaluated were linalool, α -terpeniol, citronellol, nerol, and geraniol (Table 1). Terpene alcohol precursors can be glycosidically conjugated forms or polyhydroxylated forms (28). In most cases, the glycosidically bound form is the most abundant (28). In this study, the concentrations of the aglycones for most of the terpene alcohol were similar to the free form, ranging from ng/kg to mg/kg levels. However, the concentrations of bound rose oxide, linalool oxide, and hydroxylinalool were much higher than the free form reported previously (23). The precursor of rose oxide was approximately 10 times that in the free form. The linalool oxide precursor form was 100–1000 times the free form, while hydroxylinalool glycosides were 2–20 times the levels of the free form, depending on the cultivar.

The overall distribution of terpene alcohol glycosides among the genotypes in the 'Marion' pedigree was similar to the distribution of the free form (23). 'Marion' had almost the complete terpene alcohol aglycone spectrum; however, the concentration of most terpene alcohol aglycone was very low, except for linalool oxide and hydroxylinalool. The content of total terpene alcohol glycosides in 'Marion' was approximately 4000 $\mu\text{g/kg}$. The cultivars representing the 'Marion' maternal side, including 'Chehalem', 'Santiam', and 'Himalaya', had similar total amounts of terpene alcohol precursors with concentrations ranging from 3300 to 4500 $\mu\text{g/kg}$. The cultivars representing the 'Marion' paternal side, including 'Olallie' and 'Logan', contained much higher terpene alcohol precursor levels, with concentrations varying from 4000 to 8000 $\mu\text{g/kg}$. 'Olallie' and 'Logan' had extremely high levels of linalool and linalool oxide precursors. 'Meeker', as a raspberry representative, had the lowest terpene alcohol glycosides, only 1500 $\mu\text{g/kg}$ in fruit from the 2008 growing season and 2000 $\mu\text{g/kg}$ for the 2007 season, with only a trace level of linalool oxide and no detectable rose oxide.

Linalool has been reported as one of the most important aroma compounds in blackberries (12–14, 29). The distribution of linalool glycosides was similar to the distribution of the free form linalool, as was the distribution of hydroxylinalool. In the 'Marion' pedigree, the cultivars representing the maternal side, including 'Chehalem', 'Santiam', and 'Himalaya', had low levels of these compounds. The content of linalool in these three cultivars was 40–180 $\mu\text{g/kg}$, while hydroxylinalool was 800–2200 $\mu\text{g/kg}$. The cultivars representing the paternal side had a much higher amount, with concentrations ranging from 1000 to 2400 and 2000–5000 $\mu\text{g/kg}$ for linalool and hydroxylinalool, respectively. 'Marion' was in between these values and had a content of 150–170 and 1800–2600 $\mu\text{g/kg}$ for linalool and hydroxylinalool, respectively. The pattern suggested that linalool and hydroxylinalool were highly heritable in blackberry.

The concentration of hydroxylinalool glycoside was 100 times higher than the free form. Hydroxylinalool is considered as the major transformation product of linalool (30). The β -D-glucoside of hydroxylinalool has been found in berry fruit (31). It was interesting to note that 'Marion' had an intermediate level of (*E*)-linalool oxide precursor as compared to its 'Chehalem' and 'Olallie' parents. 'Chehalem', in turn, had an intermediate level of (*E*)-linalool oxide precursor as compared to its parents 'Santiam' and 'Himalaya', while 'Olallie' was also intermediate to its parents in levels of the (*E*)-linalool oxide precursor. The results also suggested that the linalool oxide precursor was predictably inherited in these blackberries.

Norisoprenoids. The norisoprenoids can be formed by direct degradation of carotenoids, and they also frequently occur as bound forms in fruits (15, 31). The bound glycosidically conjugated norisoprenoids can be mono-oxygenated conjugates, dioxygenated conjugates, or higher-oxygenated conjugates (8). In this study, dihydro- α -ionol, megastigmatrienone, 4-oxo-7,8-dihydro- β -ionol, and 3-hydroxy-7,8-dihydro- β -ionol were only found in precursor form, while there was no detectable dihydro- β -ionone and α -ionone, and only a trace level of β -ionone was found, possibly formed from acid and/or enzyme hydrolysis of the precursors. It has been described that 3-hydroxy-7,8-dihydro- β -ionol could serve as a precursor of damascenone under acidic conditions (32). As compared to the free form, concentrations of the aglycons such as α -ionol, dihydro- β -ionol, 4-hydroxy- β -ionone, 3-oxo- α -ionol, and 4-oxo- β -ionol were up to 20 times higher for some cultivars.

The distribution of norisoprenoid precursors in the genotypes in the 'Marion' pedigree was similar to those for the free form. 'Marion' had a moderate level of norisoprenoid precursors, with a total concentration around 130 $\mu\text{g/kg}$. The cultivars representing the maternal side of the pedigree, including 'Chehalem' and 'Himalaya', only had a trace level of norisoprenoid precursors, around 20–30 $\mu\text{g/kg}$, while the cultivars representing the paternal side of the pedigree, including 'Olallie', 'Logan', and 'Meeker', had a much higher amount, with concentrations ranging from 160 to 1200 $\mu\text{g/kg}$. 'Meeker' had an extremely high concentration of norisoprenoid precursors, 650 $\mu\text{g/kg}$ for the berry samples from the 2008 growing season and 1200 $\mu\text{g/kg}$ for the 2007 season. The major norisoprenoid glycoside in 'Meeker' was α -ionol, and its concentration ranged from 370 to 880 $\mu\text{g/kg}$. 'Santiam' also had a relatively high level of α -ionol.

The major norisoprenoid aglycons, including α -ionol, 3-oxo- α -ionol, 4-oxo- α -ionol, 4-oxo-7,8-dihydro- β -ionol, and 3-hydroxy-7,8-dihydro- β -ionol, had a similar distribution pattern. The cultivars from the 'Marion' maternal side had much lower concentrations than those from the paternal side, while 'Marion' had an intermediate level. Because 'Meeker' red raspberry had a uniquely high level of the norisoprenoid precursor, the distribution pattern of these compounds among the 'Marion' pedigree suggested that the major norisoprenoids in 'Marion' might be inherited from the raspberry cultivar in its pedigree.

Shikimic Acid Derivatives. The amount of aglycones of shikimic acid derivatives was 2–5 times the free form. Phenylmethanol and 2-phenylethanol had extremely high levels of precursor forms, with concentrations of 1200–7000 and 170–1000 $\mu\text{g/kg}$, respectively, 3 and 5 times higher than the free form. The presence of these two compounds in blackberries and raspberries and in precursor hydrolysates has been previously reported (15, 31). All of the cultivars in the 'Marion' pedigree had a similar content of shikimic acid derivatives.

Although a high amount of shikimic acid derivatives existed in the 'Marion' pedigree, the sensorial contribution of shikimic acid derivatives to berry fruit is probably very small, since many of

Table 1. Volatile Precursors in Fruit Harvested in 2007 and 2008 from the Genotypes in the Pedigree of 'Marion' Blackberry ($\mu\text{g/kg} \pm \text{SD}$)^a

LRI (wax)	compounds	year	'Marion'	'Chehalem'	'Santiam'	'Himalaya'	'Ollalie'	'Logan'	'Meeker'
	terpene alcohols	2007	4068	3427	3653	4039	7510	6381	1959
		2008	3719	4435	4517	3340	4179	8133	1574
1341	<i>trans</i> -rose oxide ^c	2007	21 \pm 2 bA	15 \pm 2 bA	120 \pm 10 dA	ND	16.7 \pm 0.7 bA	100 \pm 7 cA	ND
		2008	23 \pm 2 bA	13 \pm 1 bA	110 \pm 10 cA	ND	23 \pm 4 bB	92 \pm 7 cA	ND
1354	<i>cis</i> -rose oxide ^c	2007	9.4 \pm 0.1 bA	ND	45 \pm 3 cA	ND	7.0 \pm 0.5 bA	45 \pm 2 cB	ND
		2008	8.1 \pm 0.5 bA	ND	41 \pm 4 dA	ND	8.6 \pm 0.1 bA	33 \pm 3 cA	ND
1425	<i>trans</i> -furanic-linalool oxide	2007	1110 \pm 90 dA	1710 \pm 90 eA	220 \pm 30 bA	620 \pm 80 cA	590 \pm 70 cA	930 \pm 90 dA	30 \pm 3 aA
		2008	1500 \pm 200 dA	1960 \pm 90 eA	240 \pm 40 bA	520 \pm 10 bcA	700 \pm 100 cA	1650 \pm 40 eB	65 \pm 10 aB
1451	<i>cis</i> -furanic-linalool oxide	2007	100 \pm 10 bA	310 \pm 30 cA	45 \pm 6 aA	500 \pm 60 dA	58 \pm 7 aA	120 \pm 20 bA	51 \pm 3 aA
		2008	150 \pm 30 bA	300 \pm 30 cA	33 \pm 2 aA	380 \pm 80 dA	67 \pm 10 aA	137 \pm 6 bA	71 \pm 3 aB
1532	linalool	2007	173 \pm 4 bA	43 \pm 4 aA	87 \pm 7 aA	73 \pm 9 aA	1560 \pm 90 dB	1320 \pm 40 cA	110 \pm 10 abB
		2008	154 \pm 6 bA	51 \pm 1 aA	177 \pm 6 bB	52 \pm 4 aA	1060 \pm 20 cA	2400 \pm 90 dB	73 \pm 2 aA
1577	4-terpineol	2007	1.8 \pm 0.4 aA	6.8 \pm 0.5 bA	14 \pm 1 cA	82 \pm 2 eB	1.5 \pm 0.001 aA	2.0 \pm 0.001 aA	24 \pm 4 dB
		2008	2.7 \pm 0.1 aB	8.2 \pm 0.1 bB	28 \pm 2 cB	57 \pm 1 dA	1.4 \pm 0.001 aA	3.8 \pm 0.2 aB	11 \pm 1 bA
1672	1,8-menthadien-4-ol ^c	2007	7.1 \pm 0.2 bA	19 \pm 1 cA	39 \pm 1 dA	82 \pm 8 eA	ND	ND	ND
		2008	10 \pm 1 bB	23 \pm 2 cA	61 \pm 2 dB	70 \pm 10 eA	ND	ND	ND
1682	borneol	2007	2.7 \pm 0.2 aA	24 \pm 1 cA	160 \pm 10 fA	91 \pm 5 eB	11.4 \pm 0.5 bcB	6.3 \pm 0.5 abA	56 \pm 9 dB
		2008	2.4 \pm 0.5 aA	34 \pm 1 cB	310 \pm 10 eB	63 \pm 2 dA	7.6 \pm 0.8 abA	20 \pm 2 bB	6.8 \pm 0.5 abA
1684	α -terpineol	2007	6.8 \pm 0.1 aA	70 \pm 2 bA	71 \pm 1 bA	560 \pm 30 eB	225 \pm 1 dB	204 \pm 4 dA	120 \pm 10 cB
		2008	6.5 \pm 0.7 aA	90 \pm 2 bcB	128 \pm 9 cB	430 \pm 30 dA	143 \pm 4 cA	570 \pm 40 eB	38 \pm 1 bA
1769	citronellol	2007	3.2 \pm 0.01 aA	7.7 \pm 0.2 bA	30 \pm 1 cA	4.4 \pm 0.1 aA	9.1 \pm 0.1 bA	4.7 \pm 0.1 aA	4.6 \pm 0.2 aB
		2008	3.2 \pm 0.6 aA	7.2 \pm 0.2 aA	57 \pm 6 bB	4.9 \pm 0.1 aA	9.4 \pm 0.2 aA	8.7 \pm 0.2 aB	3.4 \pm 0.01 aA
1794	myrtenol ^{b,c}	2007	15 \pm 2 bcA	26.6 \pm 0.5 cA	146 \pm 4 eA	190 \pm 20 fA	3.2 \pm 0.1 aA	12.7 \pm 0.6 bA	113 \pm 6 dB
		2008	15.2 \pm 0.2 bA	34 \pm 2 cB	170 \pm 20 eA	150 \pm 20 dA	5.1 \pm 0.1 aB	19 \pm 2 bcB	49 \pm 2 cA
1801	nopol	2007	7.6 \pm 1 abA	47 \pm 1 cA	114 \pm 4 dA	380 \pm 30 eB	1.3 \pm 0.1 aA	4.2 \pm 0.1 aA	12.0 \pm 0.6 bA
		2008	8.9 \pm 0.1 abA	62 \pm 6 cB	162 \pm 5 dB	240 \pm 30 eA	3.0 \pm 0.1 aB	11 \pm 1 bB	15 \pm 1 bB
1810	nerol	2007	19.0 \pm 0.2 aA	25.3 \pm 0.1 abA	49 \pm 2 bcA	17.7 \pm 0.6 aA	75 \pm 1 dB	63 \pm 1 cdA	78 \pm 2 dB
		2008	17.7 \pm 0.8 aA	27.8 \pm 0.5 bA	69.6 \pm 0.1 dB	18 \pm 1 aA	54 \pm 1 cA	170 \pm 4 eB	49 \pm 1 cA
1821	isogeraniol ^{b,d}	2007	2.8 \pm 0.2 aA	10.6 \pm 0.1 bcA	76 \pm 3 eA	11 \pm 1 cA	6.6 \pm 0.2 bA	8.4 \pm 0.2 aA	15 \pm 1 dA
		2008	2.8 \pm 0.2 aA	14 \pm 1 bB	196 \pm 9 dB	11.8 \pm 0.8 bA	8.1 \pm 0.2 abB	13.3 \pm 0.8 bB	22.8 \pm 0.2 cB
1859	<i>p</i> -cymen-8-ol ^d	2007	ND	210 \pm 10 cA	25 \pm 2 bA	330 \pm 20 dA	ND	ND	ND
		2008	ND	337 \pm 2 dB	48 \pm 3 bB	270 \pm 40 cA	ND	ND	ND
1863	geraniol	2007	21.5 \pm 0.5 aA	50.6 \pm 0.4 bA	247 \pm 6 eA	61 \pm 2 bA	175 \pm 2 dA	120 \pm 4 cA	400 \pm 30 fA
		2008	27.8 \pm 0.1 aB	71 \pm 1 cB	386 \pm 2 fB	53 \pm 2 bA	199 \pm 7 dB	256 \pm 6 eB	440 \pm 10 gA
2012	perilla alcohol	2007	7.0 \pm 0.5 abA	12.0 \pm 0.6 bA	45 \pm 1 cA	147 \pm 8 dB	ND	ND	15 \pm 1 b
		2008	7 \pm 1 abA	12.7 \pm 0.1 bA	71 \pm 3 cB	120 \pm 10 dA	ND	ND	ND
2310	(<i>E</i>)-2,6-dimethylocta-2,7-diene-1,6-diol ^e	2007	2560 \pm 90 bB	840 \pm 70 aA	2120 \pm 90 bA	890 \pm 50 aA	4770 \pm 90 dB	3440 \pm 90 cB	930 \pm 80 aB
		2008	1780 \pm 90 cA	1390 \pm 90 bB	2230 \pm 90 dA	900 \pm 30 aA	1890 \pm 90 cA	2750 \pm 90 eA	730 \pm 20 aA
	norisoprenoids	2007	125	25	180	25	180	189	1186
		2008	146	28	303	21	158	227	644
1464	theaspirane A	2007	33 \pm 5 cA	1.9 \pm 0.001 aA	38 \pm 3 cA	1.8 \pm 0.001 aA	3.4 \pm 0.4 aA	8.7 \pm 0.4 bB	10.1 \pm 0.7 bB
		2008	58 \pm 10 bB	2.0 \pm 0.07 aA	43 \pm 5 bA	2.0 \pm 0.07 aA	4.1 \pm 0.1 aA	2.4 \pm 0.5 aA	3.9 \pm 0.1 aA
1500	theaspirane B	2007	32.9 \pm 0.7 dA	ND	35 \pm 2 dB	ND	1 \pm 0.1 abA	4.2 \pm 0.2 b	8.9 \pm 0.1 cB
		2008	38 \pm 0.001 dB	ND	20 \pm 2 cA	ND	1.5 \pm 0.05 abB	ND	2.5 \pm 0.2 bA
1810	β -damascenone	2007	5.9 \pm 0.001 b	6.1 \pm 0.001 bA	ND	8.0 \pm 0.1 dA	6.1 \pm 0.001 bA	6.1 \pm 0.001 bA	7.0 \pm 0.5 cA
		2008	ND	6.5 \pm 0.6 bA	ND	8.0 \pm 0.1 dA	6.2 \pm 0.001 bA	6.2 \pm 0.001 bA	7.0 \pm 0.01 cA
1908	α -ionol ^{b,f}	2007	14 \pm 1 abA	1.8 \pm 0.05 aA	61 \pm 6 cA	1.8 \pm 0.1 aA	44 \pm 2 bcA	63 \pm 3 cA	880 \pm 10 dB
		2008	15 \pm 2 abA	2.5 \pm 0.1 aB	144 \pm 5 dB	2.0 \pm 0.2 aA	42 \pm 2 bA	99 \pm 2 cB	370 \pm 20 eA
1936	β -ionone	2007	ND	ND	ND	ND	26 \pm 0.001 bA	26 \pm 0.001 bA	26.6 \pm 0.1 bA
		2008	ND	ND	ND	ND	26 \pm 0.001 bA	26.6 \pm 0.001 bA	26.6 \pm 0.1 bA
1947	dihydro- α -ionol ^{b,f}	2007	ND	ND	3.8 \pm 0.1 dA	ND	2.4 \pm 0.1 bA	3.0 \pm 0.1 cA	ND
		2008	ND	ND	7.6 \pm 0.7 cB	ND	2.8 \pm 0.5 bA	8.6 \pm 0.5 dB	ND
1964	dihydro- β -ionol ^{b,f}	2007	4.8 \pm 0.2 bA	ND	24.1 \pm 0.1 dA	ND	65 \pm 3 eB	17.7 \pm 0.8 cA	ND
		2008	4.6 \pm 0.2 bA	ND	64 \pm 3 eB	ND	34 \pm 3 cA	49 \pm 1 dB	ND
2365	megastigmatrienone ^{b,f}	2007	5.1 \pm 0.2 b	ND	8 \pm 1 cA	ND	7.3 \pm 0.2 bcA	1.5 \pm 0.1 aA	88 \pm 2 dB
		2008	ND	ND	12.2 \pm 0.7 dB	ND	8.1 \pm 0.2 cA	1.5 \pm 0.1 bA	39 \pm 6 eA
2678	4-oxo- β -ionone ^{b,f}	2007	ND	ND	ND	ND	ND	ND	4.6 \pm 0.7 A
		2008	ND	ND	ND	ND	ND	ND	6.1 \pm 0.7 A
2833	4-hydroxy- β -ionone ^{b,f}	2007	ND	ND	ND	ND	ND	30 \pm 2 b	86 \pm 7 aA
		2008	ND	ND	ND	ND	ND	ND	101 \pm 6 B
2856	3-oxo- α -ionol ^{b,f}	2007	11.6 \pm 0.1 bcA	9.9 \pm 0.5 abA	7.1 \pm 0.5 aA	7.8 \pm 0.5 aB	11.9 \pm 0.2 cA	9 \pm 1 abA	27 \pm 3 dA
		2008	11.4 \pm 0.2 cA	11 \pm 1 bcA	9 \pm 1 bA	5.6 \pm 0.5 aA	16 \pm 1 dB	11.9 \pm 0.5 cB	23 \pm 1 eA
2861	4-oxo- β -ionol ^{b,f}	2007	6.1 \pm 0.1 bA	ND	ND	ND	6.8 \pm 0.2 bA	12 \pm 1 cA	29 \pm 3 dA
		2008	6.1 \pm 0.5 bA	ND	ND	ND	8.6 \pm 0.5 bB	15.2 \pm 0.5 cB	49 \pm 2 dB
2886	4-oxo-7,8-dihydro- β -ionol ^{b,f}	2007	6.3 \pm 0.2 dA	ND	2.8 \pm 0.02 bA	ND	4.1 \pm 0.1 cA	3.3 \pm 0.1 bA	8 \pm 1 eA
		2008	7.1 \pm 0.5 dA	ND	3.0 \pm 0.03 bA	ND	4.6 \pm 0.1 cA	3.8 \pm 0.2 bcA	11.4 \pm 0.7 eB
2950	3-hydroxy-7,8-dihydro- β -ionol ^{b,f}	2007	5.1 \pm 0.2 bA	5.8 \pm 0.2 bA	ND	5.1 \pm 0.5 bB	2.5 \pm 0.1 aA	4.8 \pm 0.5 bB	11.1 \pm 0.07 cB
		2008	5.8 \pm 0.5 dA	6.6 \pm 0.5 dA	ND	3.0 \pm 0.1 bA	3.5 \pm 0.2 bB	3.5 \pm 0.1 bA	4.6 \pm 0.2 cA
	shikimic acid	2007	4364	3796	3052	2809	1982	3524	3675
	derivatives	2008	4653	5423	3845	1991	2718	5835	8247
1885	phenylmethanol	2007	3880 \pm 60 dA	2540 \pm 40 bcA	2620 \pm 7 cA	1730 \pm 50 abB	1190 \pm 70 aA	3050 \pm 30 cA	2510 \pm 50 bcA
		2008	4200 \pm 40 dB	3930 \pm 50 dB	3170 \pm 20 cB	1270 \pm 40 aA	1790 \pm 30 bB	4940 \pm 90 eB	6970 \pm 90 fB

Table 1. Continued

LRI (wax)	compounds	year	'Marion'	'Chehalem'	'Santiam'	'Himalaya'	'Olallie'	'Logan'	'Meeker'
1920	2-phenylethanol	2007	253 ± 5 bA	870 ± 90 eA	146 ± 2 aA	790 ± 80 dB	520 ± 60 cA	210 ± 10 abA	170 ± 20 aA
		2008	260 ± 6 aA	1000 ± 30 cA	170 ± 10 aA	520 ± 50 bA	720 ± 90 bA	300 ± 20 aA	230 ± 20 aA
2010	4-phenyl-2-butanol	2007	34 ± 4 bB	170 ± 10 dA	42 ± 5 bA	2.7 ± 0.1 aB	70 ± 2 cB	2.53 ± 0.06 aA	ND
		2008	13.9 ± 0.5 bA	191 ± 7 eA	85 ± 7 dB	1.1 ± 0.1 aA	37 ± 1 cA	5.8 ± 0.7 abB	ND
2119	cumic alcohol	2007	ND	7.0 ± 0.1 bA	2.9 ± 0.2 aA	32 ± 2 cB	ND	ND	118 ± 4 dB
		2008	ND	7.2 ± 0.4 cA	3.0 ± 0.1 bA	18 ± 2 eA	ND	ND	14 ± 1 dA
2303	cinnamyl alcohol	2007	39.2 ± 0.6 dB	29 ± 2 bA	32 ± 1 bcA	34 ± 1 cB	80 ± 1 eB	33 ± 2 cA	14 ± 1 aA
		2008	25 ± 3 aA	34 ± 3 abA	39 ± 2 bB	27.8 ± 0.5 aA	43 ± 3 bA	30 ± 1 aA	26 ± 4 aB
1755	methyl salicylate	2007	76 ± 2 eA	78 ± 1 eA	78 ± 2 eA	58 ± 1 cB	24 ± 1 aA	72 ± 1 dA	42 ± 2 bA
		2008	78 ± 2 dA	115 ± 2 fB	100 ± 2 eB	38 ± 1 bA	29 ± 1 aB	115 ± 3 fB	53 ± 1 cB
2182	eugenol	2007	23 ± 1 aB	26.6 ± 0.4 aA	53 ± 2 bA	22 ± 1 aA	18 ± 1 aA	33 ± 1 abA	620 ± 20 cA
		2008	11.6 ± 0.7 aA	37 ± 1 bB	158 ± 3 dB	19 ± 1 abA	15 ± 1 abA	133 ± 7 cB	730 ± 20 eB
2360	chavicol ^{b,g}	2007	24 ± 1 bA	16.5 ± 0.4 abA	10 ± 1 aA	15.2 ± 0.1 abB	12.2 ± 0.6 aA	25 ± 2 bA	139 ± 7 cA
		2008	29 ± 4 bA	22 ± 2 bB	20 ± 1 bB	10.1 ± 0.7 aA	13 ± 1 aA	29 ± 1 bA	160 ± 10 cA
2364	isoeugenol	2007	1.0 ± 0.04 aA	0.9 ± 0.07 aA	19.0 ± 0.3 dA	20.3 ± 0.3 dA	13.9 ± 0.1 bcA	12.3 ± 0.1 bA	15 ± 1 cB
		2008	1.3 ± 0.1 aA	1.4 ± 0.1 aB	22.8 ± 0.3 cB	25 ± 1 cB	15.2 ± 0.1 bB	97 ± 4 dB	2.5 ± 0.1 aA
2571	methoxyeugenol ^{b,g}	2007	34 ± 2 aA	58 ± 2 dA	49 ± 2 bcA	105 ± 4 fB	54 ± 2 cdA	86 ± 4 eA	47 ± 6 bA
		2008	34 ± 3 aA	85 ± 3 cB	77 ± 2 cB	62 ± 7 bA	56 ± 2 bA	185 ± 9 dB	62 ± 4 bA
	alcohols	2007	7521	3547	1570	1479	3749	1094	6426
		2008	7694	3382	1466	1509	2356	1521	3067
1355	hexan-1-ol	2007	1390 ± 90 dA	980 ± 90 cA	460 ± 70 bA	730 ± 50 cA	340 ± 30 abB	80 ± 3 aA	2870 ± 90 eB
		2008	1460 ± 90 dA	850 ± 90 cA	430 ± 10 abA	530 ± 90 bA	220 ± 50 abA	73 ± 3 aA	540 ± 30 bA
1379	(Z)-hex-3-en-1-ol	2007	2410 ± 90 eB	1420 ± 90 cA	470 ± 40 bB	87 ± 9 aA	250 ± 50 abA	440 ± 60 bA	1760 ± 90 dB
		2008	1790 ± 90 cA	1440 ± 90 cA	293 ± 6 aA	81 ± 4 aA	260 ± 50 aA	430 ± 40 abA	780 ± 80 bA
1400	(E)-hex-2-en-1-ol	2007	450 ± 30 eA	230 ± 20 cA	200 ± 30 bcB	152 ± 8 bA	320 ± 20 dA	75 ± 9 aA	92 ± 9 a
		2008	330 ± 50 cdA	210 ± 30 bcA	82 ± 3 aA	190 ± 10 bB	350 ± 30 dA	85 ± 6 aA	ND
1326	heptan-2-ol	2007	3030 ± 90 cA	450 ± 50 bA	100 ± 10 bA	480 ± 50 bA	2710 ± 90 cB	360 ± 60 bA	ND
		2008	3840 ± 90 dB	410 ± 60 bA	220 ± 30 bB	490 ± 50 bA	1420 ± 90 cA	510 ± 10 bB	ND
1446	oct-1-en-3-ol	2007	6.3 ± 0.4 dA	7 ± 1 dA	1.0 ± 0.1 aA	4.0 ± 0.2 cA	2.5 ± 0.1 aA	3.9 ± 0.1 cA	2.8 ± 0.6 bA
		2008	9 ± 1 cB	10 ± 1 cB	1.4 ± 0.1 aA	3.7 ± 0.4 bA	3.8 ± 0.2 bB	5.4 ± 0.1 bB	5.4 ± 0.2 bB
1452	heptan-1-ol	2007	80 ± 10 cA	250 ± 20 eA	150 ± 10 dA	34 ± 2 ab	25 ± 1 a	65 ± 8 bcA	32 ± 4 ab
		2008	81 ± 7 bA	250 ± 20 dA	150 ± 30 cA	ND	ND	81 ± 3 bA	ND
1461	6-methylhept-5-en-2-ol	2007	120 ± 10 bA	170 ± 10 cA	60 ± 8 aA	43 ± 5 aA	76 ± 9 aA	57 ± 8 aA	410 ± 30 dA
		2008	150 ± 20 bA	150 ± 20 bA	48 ± 2 aA	40 ± 2 aA	77 ± 9 aA	54 ± 1 aA	360 ± 20 cA
1543	octan-1-ol	2007	19 ± 1 cA	30 ± 2 dA	75 ± 3 eA	86 ± 7 fB	15 ± 1 bA	6.2 ± 0.2 aA	20 ± 1 cA
		2008	22 ± 2 bA	53 ± 2 dB	160 ± 5 eB	44 ± 1 cA	14 ± 1 aA	10.1 ± 0.5 abB	19 ± 2 bA
2060	Furaneol	2007	3260 ± 90 dA	210 ± 8 aA	770 ± 30 cA	4830 ± 90 eA	3070 ± 90 dB	220 ± 2 aA	480 ± 6 bA
		2008	2750 ± 90 dA	225 ± 2 aA	840 ± 20 bA	4840 ± 90 eA	2060 ± 90 cA	224 ± 2 aA	640 ± 10 bB

^a LRI, linear retention index. Letters a–g within the rows indicated the significant difference of the compounds among the cultivars by ANOVA with Tukey's test at $p = 0.05$. Letters A and B between years indicated the significant difference between years by t test. ^b Tentatively identified by MS and LRI from literature. ^c The concentration was estimated by the compound 4-terpineol in the SBSE method. ^d The concentration was estimated by the compound geraniol in the SBSE method. ^e The concentration was estimated by the compound isoeugenol in the SPE method. ^f The concentration was estimated by the compound β -ionone in the SBSE method. ^g The concentration was estimated by the compound isoeugenol in the SBSE method.

these compounds had low concentrations and high sensory thresholds. The distribution pattern of shikimic acid derivatives in the 'Marion' pedigree could be less important.

Alcohols. The glycosides of hexanol and (Z)-hex-3-en-1-ol have been identified in plant tissue (15, 31). 'Marion' had the highest amount of C6 alcohol glycosides, with a concentration of 3600 $\mu\text{g}/\text{kg}$ for the berry samples from the 2008 growing season and 4300 $\mu\text{g}/\text{kg}$ for 2007. 'Meeker' red raspberry also had a high concentration of the aglycones of the C6 compounds, 1500 $\mu\text{g}/\text{kg}$ for the berry samples from the 2008 growing season and 4700 $\mu\text{g}/\text{kg}$ for 2007, although it had a low level of (E)-hex-2-en-1-ol.

'Marion' also had the highest level of 2-heptanol glycoside, with the concentration ranging from 3000 to 3800 $\mu\text{g}/\text{kg}$; its parent 'Olallie' contained a similar 2-heptanol level. All other cultivars had a much lower level of 2-heptanol glycoside. 2-Heptanolglycoside was not detected in 'Meeker' red raspberry. 2-Heptanol has been identified as an important aroma contributor in blackberries (12–14, 29).

Furanones. The glycosidically bound Furaneol has been a reported in many fruits (31)(33)(34, 35) in the forms of β -D-glucopyranoside and 6'-O-malonyl- β -D-glucopyranoside (36). Similar to the distribution of the free Furaneol in the genotypes representing the 'Marion' pedigree, 'Marion', 'Himalaya', and

'Olallie' had much higher Furaneol glycoside, with concentrations ranging from 2000 to 4800 $\mu\text{g}/\text{kg}$. The concentration of Furaneol glycoside in all other cultivars was much lower, only 210–840 $\mu\text{g}/\text{kg}$, which was similar to the free form. Furaneol can be converted to mesifurane through methylation by O-methyltransferase (37). Mesifurane is present only as the free form.

In conclusion, the most abundant volatile precursors in the 'Marion' pedigree genotypes were alcohols, followed by shikimic acid derivatives. High amounts of furanone glycosides were also detected, while norisoprenoids only existed in a small amount in blackberries. The volatile precursor composition in the fruit from the genotypes in the 'Marion' pedigree was very similar to their free volatile distribution. Among the genotypes representing the 'Marion' pedigree, the concentration of most volatile precursors in the offspring was intermediate to the levels of the parents, although in some cases, the concentration of volatile precursors in the offspring exceeded the parents, behaving as transgressive segregants.

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